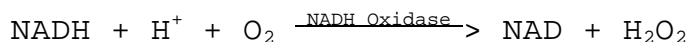


Enzymatic Assay of NADH OXIDASE

PRINCIPLE:



CONDITIONS: T = 30°C, pH = 7.0, A_{340nm}, Light path = 1 cm

METHOD: Continuous Spectrophotometric Rate Determination

REAGENTS:

- A. 250 mM Potassium Phosphate Buffer, pH 7.0 at 30°C
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379. Adjust to pH 7.0 at 30°C with 1 M KOH.)
- B. 1 mM Flavin Adenine Dinucleotide Solution (FAD)
(Prepare 10 ml in deionized water using Flavin Adenine Dinucleotide, Disodium Salt, Sigma Prod. No. F-6625.)
- C. 2.0 mM β-Nicotinamide Adenine Dinucleotide, Reduced Form Solution (NADH)
(Dissolve the contents of one 10 mg vial of β-Nicotinamide Adenine Dinucleotide, Reduced Form, Sigma Stock No. 340-110, in the appropriate volume of deionized water. **PREPARE FRESH.**)
- D. 30 mM Potassium Phosphate Buffer with 0.1% (w/v) Bovine Serum Albumin, pH 7.5 at 30°C (Enzyme Diluent)
(Prepare 100 ml in deionized water using Potassium Phosphate, Monobasic, Anhydrous, Sigma Prod. No. P-5379, and Albumin, Bovine, Sigma Prod. No. A-4503. Adjust to pH 7.5 at 30°C with 1 M KOH.)
- E. NADH Oxidase Enzyme Solution
(Immediately before use, prepare a solution containing 0.2 unit/ml of NADH Oxidase in cold Reagent D.)

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PROCEDURE:

Pipette (in milliliters) the following reagents into suitable cuvettes:

| | <u>Test</u> | <u>Blank</u> |
|--------------------|-------------|--------------|
| Deionized Water | 1.90 | 1.90 |
| Reagent A (Buffer) | 0.60 | 0.60 |
| Reagent B (FAD) | 0.30 | 0.30 |
| Reagent C (NADH) | 0.10 | 0.10 |

Mix by inversion and equilibrate to 30°C. Monitor the $A_{340\text{nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then add:

| | | |
|-----------------------------|-------|-------|
| Reagent E (Enzyme Solution) | 0.10 | ----- |
| Reagent D (Enzyme Diluent) | ----- | 0.10 |

Immediately mix by inversion and record the decrease in $A_{340\text{nm}}$ for approximately 5 minutes. Obtain the $\Delta A_{340\text{nm}}/\text{minute}$ using the maximum linear rate for both the Test and Blank.

CALCULATIONS:

$$\text{Units/ml enzyme} = \frac{(\Delta A_{340\text{nm}}/\text{min Test} - \Delta A_{340\text{nm}}/\text{min Blank})(3)(\text{df})}{(6.22)(0.10)}$$

3 = Total volume (in milliliters) of assay

df = Dilution factor

6.22 = Millimolar extinction coefficient of β -NADH at 340 nm

0.10 = Volume (in milliliter) of enzyme used

$$\text{Units/mg solid} = \frac{\text{units/ml enzyme}}{\text{mg solid/ml enzyme}}$$

$$\text{Units/mg protein} = \frac{\text{units/ml enzyme}}{\text{mg protein/ml enzyme}}$$

UNIT DEFINITION:

One unit will oxidize 1.0 μmole of β -NADH per minute at pH 7.0 at 30°C.

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FINAL ASSAY CONCENTRATIONS:

In a 3.00 ml reaction mix, the final assay concentrations are

51 mM potassium phosphate, 0.07 mM β -nicotinamide adenine dinucleotide, reduced form, 0.1 mM flavin adenine dinucleotide, 0.003% (w/v) bovine serum albumin, and 0.02 unit NADH oxidase.

REFERENCE:

Reusch, V.M., Burger, M.M. (1974) *Journal of Biological Chemistry* **249**, 5337-5345

NOTES:

1. This assay is based on the cited reference.
2. Where Sigma Product or Stock numbers are specified, equivalent reagents may be substituted.

This procedure is for informational purposes. For a current copy of Sigma's quality control procedure contact our Technical Service Department.